C2-1137

Genomic Comparison of USA300 and USA400 MRSA Strains by Optical Mapping

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ABSTRACT

Introduction

Genomes of *Staphylococcus aureus* primarily evolve due to insertion of pathogenicity and/or genomic islands, which are known to harbor many virulence genes. However, not all MRSA strains have the same set of these islands. Optical mapping is a novel tool that creates high-resolution ordered restriction maps of whole bacterial genomes. These whole genome optical maps can be used as a discovery tool to identify genomic rearrangements such as deletions, insertions and duplication when compared with sequenced and annotated strains of the same species. We tested this possibility by comparing optical maps of methicillin-resistant *S. aureus* (MRSA) strains belonging to two major community-associated MRSA clones in the United States.

Methods

Strains: A total of eight strains, two from the USA300 clone (FPR3757 and NRS-384), four from the USA400 clone (MW2, WI-33, WI-34, and WI-99) and two additional strains, WI-23 (a clonally unrelated strain) and WI-184-S (a hypervirulent MSSA strain) were chosen. Optical maps of these strains were generated by spreading their chromosomes on derivatized glass surfaces followed by *in situ* digestion with *Xba*I. Digested DNA on the optical surface was stained with YOYO-1. Length and order of the restricted fragments sizes were determined by fluorescence microscopy and high-resolution digital photography. The optical maps were assembled by overlapping contiguous sets of 600-700 restriction fragments across the ~2.8 million base pairs of each genome. For the genomes already sequenced (e.g. FPR3757 and MW2), *in silico* maps were also created from sequences available from NCBI sequence databases. All of the maps were compared using the MapViewer® program (OpGen Inc).

Results

Comparison of the optical maps of the two USA300 strains showed that they were nearly identical except for the presence of two major regions of difference identified in the NRS-384 strain. These differences accounted for the ~34 Kbp of additional DNA sequence in the NRS-384 strain. Three of the four strains of the USA400 clone (MW2, WI-99, and WI-34) were identical in their optical maps as was expected because of their identical pulsed–field gel electrophoresis (PFGE) profile. However, strain WI-33, a clonally related strain differed from the other three USA300 strains because of the presence of a ~41 Kbp insertion in its genome. WI-23 and WI-184-S, two clonally unrelated strains respective to the MW2 strain by PFGE differed considerably in their optical maps.

Conclusion

Optical maps can be used as a discovery tool to locate the site and size of insertions or deletions from clonally related strains of a pathogen. These genomic rearrangement can be further characterized by PCR and sequencing to determine the structure/function of genes of interest.

INTRODUCTION

Staphylococcus aureus is one of the most common commensal bacterial organisms of the human population. It can be found in the anterior nares of 30 to 70% of the humans. It is also one of the most of common causes of hospital-acquired infections, causing clinical diseases in 2% of all patient admissions. In many industrialized countries the percent of *S. aureus* that are resistant to methicillin ranges from 20 to 60%. Since the mid 1990s, some clones of methicillin-resistant *Staphylococcus aureus* (MRSA) have become established in community-settings among individuals with different sets of risk factors compared to those seen in hospital-acquired infections. The reasons for *S. aureus* to become a successful commensal and pathogen is likely due to its ability to adapt and evolve by acquiring genetic elements through horizontal gene transfer.

Several approaches are available to distinguish between virulent and avirulent clones and genetic lineages of a pathogen. PFGE can be used to identify index strain(s) of an outbreak or understand the clonal relationship among strains. Multilocus sequence typing (MLST) could be used to determine the evolutionary lineages of pathogen. Microarray-based technologies could be used to determine the virulence profile of a pathogen.

Optical mapping is a novel tool that can be used to create high resolution restriction map (optical map) of a bacterial genome. The optical maps can be used to determine genome organization; identify genomic insertions and deletions in a genome when compared with an *in-silico* map of a related sequenced genome. An *in-silico* map is the restriction map created by the virtual restriction digestion (with any restriction enzyme) of a genome whose sequence is known. Here we explored this new technology to compare several clinical strains of methicillin-resistant *Staphylococcus aureus* (MRSA) at the genomic level.

METHODS

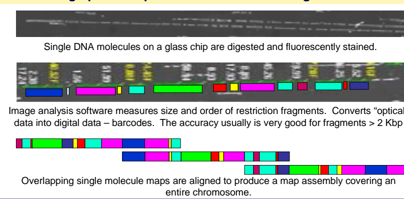
DNA isolation, restriction digestion and staining. High molecular weight DNA was isolated from each of the strains. Individual DNA molecules of were immobilized on a microfluidic device (a derivatized, negatively charged glass surface with micro capillaries. The immobilized DNAs were digested *in situ* with the restriction enzyme, *Xba*I. The restricted chromosomes were stained with a fluorescently labeled, DNA intercalating dye, YOYO-1. The stained DNAs were photographed using a fluorescent microscope interfaced with a digital camera. Image analysis was performed to quantitate the length and order of the each restriction fragment (**Figure 1**).

Whole chromosome restriction maps (optical maps) were assembled from approximately 30 individual restricted DNA molecules (**Figures 2 and 3**). An *in-silico* map was also created by the virtual restriction digest of a genome sequenced strain, MW2 using the same enzyme i.e., *Xba*I (**Figure 4**).

Optical map-based genomic relatedness was determined by the genome similarity clustering method. To generate the similarity clusters, each map was aligned to every other map. From these alignments, a pair wise percent dissimilarity was calculated by taking the total length of the unmatched regions in both genomes divided by the total size of both genomes. These dissimilarity measurements were used as inputs into the agglomerative clustering method agnes as implemented in the statistical package R. Briefly, this clustering method works by initially placing each entry in its own cluster, then iteratively joining the two nearest clusters, where the method for determining the nearest cluster was by unweighted pair-group method using arithmetic averages or UPGMA which is the average of the dissimilarities of all the points in one cluster and all the points in the other cluster.

Genome clustering was done aligning restriction fragments for local pairwise comparisons. This clustering served as an initial step in the relatedness. From these alignments, we calculated a percent identity from the fraction of the two genomes that align. These similarity scores are then passed to the statistical package R, which clusters the data using an agglomerative nesting method.

Figure 1. Generating optical maps for an entire *S. aureus* genome

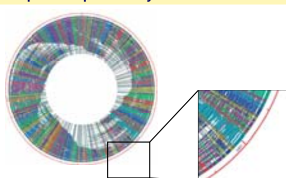


Single DNA molecules on a glass chip are digested and fluorescently stained.

Image analysis software measures size and order of restriction fragments. Converts "optical" data into digital data – barcodes. The accuracy usually is very good for fragments > 2 Kbp

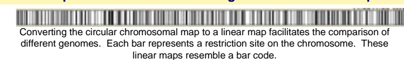
Overlapping single molecule maps are aligned to produce a map assembly covering an entire chromosome.

Figure 2. Optical map assembly



Circular bacterial genome map, showing assembly of single molecule maps and the resulting "consensus map" (outer ring).

Figure 3. Linear representation of a bacterial genome restriction map



Converting the circular chromosomal map to a linear map facilitates the comparison of different genomes. Each bar represents a restriction site on the chromosome. These linear maps resemble a bar code.

Figure 4. Comparison between optical and *in-silico* maps of the MW2 strain

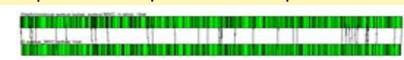
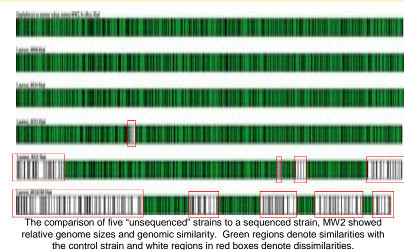


Figure 5. Pairwise Comparison of the Optical Map of the MW2 strain with Clonally Related and Unrelated *S. aureus* strains



The comparison of five "unsequenced" strains to a sequenced strain, MW2 showed relative genome sizes and genomic similarity. Green regions denote similarities with the control strain and white regions in red boxes denote dissimilarities.

Figure 6. Genomic comparison between MRSA strains two USA300-0114 strains: NRS384 and FPR3757

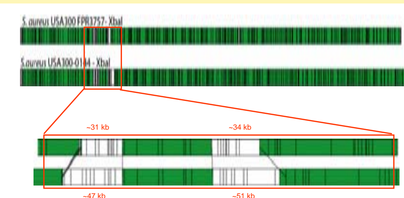
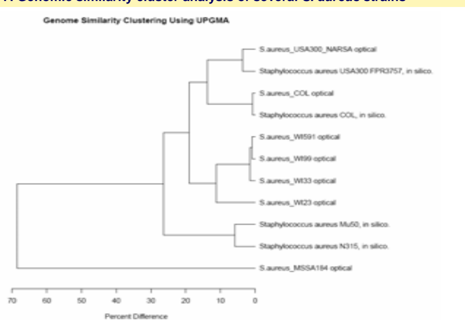


Figure 7. Genomic similarity cluster analysis of several *S. aureus* strains



RESULTS

In order to determine the quality and reliability of optical maps, the MW2 strain whose genome has been sequenced and annotated, was chosen to obtain both its optical and *in silico* map using the *Xba*I. The *in silico* map was considered the reference map. Comparison of these maps of the MW2 strain showed that the optical map was nearly identical to the *in silico* map (**Figure 4**) suggesting that these two types of maps could be compared with great confidence.

We further tested the reliability of the optical maps by comparing the optical map of the MW2 strain with two clonally related (by PFGE and MLST) MRSA strains (WI-99 and WI-34), one possibly related strain (WI-33), and two unrelated strains (WI-23 and WI-184-S) from our collection of isolates mainly collected from patients in Native American communities. We have shown earlier that strains MW2, WI-99, and WI-34 have identical PFGE and WI-23 differed from MW2 by two PFGE bands.

As expected, optical maps of MW2, WI-99, WI-34 were almost identical to each other. However, a ~41 kb in insertion was identified in the possibly related strain, WI-33 (**Figure 5**). WI-23, a clonally unrelated strain (MLST type 12) appeared very different as did the hypervirulent MSSA strain WI-184. MSSA-184 is a Panton-Valentine leukocidin negative but enterotoxin gene cluster positive strain that caused a debilitating case of necrotizing fasciitis in a diabetic patient.

Similarly, comparison of the two USA300 strains showed that they were nearly identical except for the ~34 kb additional sequence in the NRS-384 strain (**Figure 6**).

An optical map based genome similarity clustering was obtained for several clinical and reference MRSA/MSSA strains. The genome clustering showed that indeed strains such as MW2, WI-99, WI-34, WI-33 were closely related compared to non-clonal strains. As expected two USA300 strains were closely related to each other and COL was closely related to the USA300 strains than to other strains such as Mu50 or N315. Not surprisingly, MSSA strain 184 was not related to any of the strains mentioned above (**Figure 7**).

CONCLUSION

In conclusion, optical mapping appears to be a useful discovery tool that can be used to understand the genomic organization of diverse group of *S. aureus* strains with different virulence capabilities. It can also be used to determine the approximate location and size of insertions and deletions from clonally related strains on a genome. These insertions and deletions could be further characterized by PCR and sequencing to determine genes of interest including potential new virulence genes therein.

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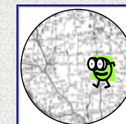
ACKNOWLEDGEMENTS

Funding for this work was provided by OpGen Inc. and Marshfield Clinic Research Foundation. Authors would like to acknowledge Adam Briska and John Henkhaus for bioinformatics support and Jennifer Brady and Mary Stemper for technical support.

"LAUGHTER, THE BEST MEDICINE"



Optical Mapping as envisioned by a 10 year old girl, Neehal Shukla